16.

The Anatomy and Morphology of the Hypophysis of Several Species of Ovo-viviparous Poeciliids.*

Hugh E. Potts

Department of Biology, Washington Square College, New York University.

(Plates I & II).

Introduction.

The constantly growing knowledge of the endocrine glands makes increasingly apparent the complex physiological interrelationships of the pituitary. A great deal has been reported both on the morphology and the physiology of the pituitary but most of the investigations have been concerned with mammals where such structures as the mammary glands, corpora lutea and placenta introduce many complicating factors. It would seem possible to obtain some clarification of the many problems arising from these studies by an investigation of the pituitary in forms such as lower vertebrates in which the presence of fewer accessory structures makes the problem less complex.

A survey of the literature has shown a surprisingly incomplete knowledge of the pituitary of lower vertebrates, especially of teleost fishes (Stendell, 1914; deBeer, 1926; Charipper, 1937). This becomes especially apparent when one considers the various reports relating cell types in the anterior pituitary with the reproductive cycle in mammals (Rasmussen, 1921; Wolfe & Cleveland, 1933, 1933; Charipper & Haterius, 1932; Kirkman, 1937) as compared with the meager literature of a similar nature concerning the bony fishes. With regard to the latter group, Matthews (1936) has shown that the pituitary gland of *Fundulus* is not static but undergoes variations in cell type distribution which may be related to the different seasons of the year. Cardoso (1934), also working on an oviparous form, *Pimelodus clarias*, has shown a relation between gonad size and injection of hypophyseal suspensions. Houssay (1931), investigating the ovo-viviparous teleost, *Cnesterodon decemmaculatus*, described a relationship between ovulation and secretions of the pituitary. In addition, Rojas et al. (1934) showed cyclic changes in the pituitary of *Jenynsia lineata*, another ovoviviparous teleost. Further, the Teleostei as a group seem to offer many opportunities for descriptive investigations correlating variable hypophyseal histology and cytology to reproductive phenomena. In this group are a vast number of diminutive tropical fishes which present many evolutionary phases of reproduction from oviparity to true viviparity. This group of viviparous fishes are of particular interest because of the relatively short and usually regular reproductive cycle. These animals may be kept in large numbers in small aquaria, assuring a plentiful supply throughout the year. In addition, most of them can be bred readily so that animals in definite stages of the reproductive cycle may be easily obtained at all times.

With these facts in mind an investigation of the morphology and histology of the pituitaries of males and females of six species of ovo-viviparous teleosts was undertaken and forms the basis of the present report. The particular species examined were carefully chosen for availability and the ease with which they could be maintained and bred under laboratory conditions. In addition all these forms showed regular reproductive cycles of approximately thirty days. It is proposed that these descriptions constitute the beginning of what should prove to be an exhaustive survey and furnish a basis for future experimental investigations.

I wish to take this opportunity to express my deep appreciation to Dr. Harry A. Charipper for his generous and helpful assistance throughout the course of this investigation.

Materials and Methods.

The pituitaries of six different species of ovo-viviparous poeciliids, *Platypoecilus variatus*, *Xiphophorus helleri* (red, and

* Accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, New York University.

green varieties), *Lebistes reticulatus*, *Limia tricolor*, *Mollienisia sphenops* and *Mollienisia latipinna* (black variety) were examined histologically. Specimens of *Mollienisia sphenops* and *latipinna* were obtained from a local dealer during the months of December, January and February. These were killed and fixed within two weeks of purchase. The other species were reared in aquaria at temperatures between 21 and 27 degrees centigrade and sacrificed at various times throughout the year.

Each fish was anaesthetized in ice water and the head severed immediately posterior to the gills and also cut just in front of the eyes. A fixing fluid composed of 18.5 cc. basic Zenker's to 1.5 cc. formalin gave the best results in about twenty hours. Following fixation, tissues were decalcified in phloroglucin 1½ hours and washed in running water 6 to 12 hours before dehydration and embedding.

Whole heads, or brain and pituitary, dissected free of all cartilage were then dehydrated and embedded in hard paraffin or tissue mat, after which they were cut at 5 micra in median sagittal, cross and horizontal sections.

The Masson technic was used almost exclusively. Timing and washing were standardized as carefully as possible in order to get comparable results on all six species. Earlier preparations were stained in Masson A ten minutes, rinsed in distilled water and destained in 1% phosphomolybdic acid 1¼ hours. They were then transferred directly to Masson C for thirty minutes, dipped in 95% alcohol, absolute alcohol, half and half absolute alcohol and xylol and then pure xylol, and finally mounted in Canada balsam. In later preparations the staining times were changed to three minutes in Masson A, thirty minutes in phosphomolybdic acid and 1 hour in Masson C. The latter timing gave better results although the staining affinities did not seem to be critically affected.

Description.

The pituitaries of the six species of poeciliids in this investigation show such striking similarities in morphology and histology that a single description will be made, using *Platypoecilus variatus* as the standard. The description will deal first with morphology and then with histology, under the headings cerebral portion (pars nervosa) and epithelial portion (pars anterior, intermedia, and uebergangsteil). Similarities and differences of the other species will then be considered separately.

I. *Platypoecilus variatus*

A. Morphology.

The pituitary is an ovoid gland, immediately behind the optic chiasma, attached to the midventral floor of the diencephalon by a short hollow stalk. There is no sella turcica but the pituitary protrudes into a depression, the hypophyseal fenestra, closed ventrally by connective tissue (Pl. II, Fig. 12).

An extremely thin meninx primitiva extends from the brain down over the stalk and over the pituitary, carrying with it numerous small blood vessels.

There are four portions of the gland, distinguishable by staining reactions and cell types, namely, pars nervosa, anterior, intermedia and uebergangsteil (Pl. II, Fig. 7). No distinct septa separate the portions. The pars nervosa is a thickened and modified portion of the floor of the diencephalon, occupying the central dorsal region of the gland. The pars anterior, like a half sphere, has its posterior surface in contact with all the other parts of the gland. In its median dorsal portion, this contact is with the nervosa. Surrounding the nervosa completely ventrally and laterally, but only its ventral half anteriorly and posteriorly, is a convoluted layer of cells, the uebergansteil, which contacts the posterior face of the pars anterior ventral and lateral to the nervosa. The pars intermedia is a shell-like portion surrounding the uebergangsteil completely ventrally, laterally and posteriorly, forming in the latter region the blunt posterior end of the gland. Anteriorly it is contiguous with the pars anterior lateral and ventral to the contact of the latter with the uebergangsteil.

B. Histology

1. Cerebral Portion (Pars Nervosa)

The cerebral portion is composed of tissue from the floor of the diencephalon. It consists of masses of neuroglia cells and many interlacing fibres continuous through the infundibular stalk with the brain (Pl. I, Figs. 2, 4). The outer walls of the stalk are covered by a thin meninx primitiva whereas the inner walls are composed of ependyma cells which line the infundibular cavity.

The nuclei of ependyma and neuroglia cells are essentially similar, being rounded to ovoid and varying considerably in size. Each is surrounded by a well defined membrane and contains a centrally located, red-staining nucleolus and scattered pink to violet chromatin material. Nuclei are more abundant near the stalk and sparser in the central and more distal parts of the nervosa.

The cytoplasm of the ependyma cells is pale gray. Many irregular processes from them extend into the infundibular cavity. The cytoplasm of the neuroglia cells is indistinct and loosely fibrous, also staining a pale gray. Many tracts of fibres extend from the central portion of the nervosa into other parts of the gland as compact processes enclosing blood vessels which are thus distributed to the epithelial portions of the gland.

Penetration by these processes is especially heavy in the uebergangsteil and intermedia (Pl. I, Fig. 6; Pl. II, Fig. 7). In the dorsal posterior region of the nervosa the fibres run caudad through the low posterior end of the basket-shaped uebergangsteil and ramify among the cells of the pars intermedia (Pl. II, Fig. 7).

Colloid bodies of varying size and shape are characteristically present in the nervosa in varying amounts (Pl. I, Fig. 6; Pl. II, Figs. 7, 11). In the central region they are usually fairly large bodies but in the posterior region they are generally fine and granular. Their staining reaction varies in different glands from violet to pink, and both colors may be present in the same gland. In some cases large basophilic masses are filled with brilliant red-staining globules.

Free single cells, generally basophiles, are often found well within the nervosa. In some cases they appear normal but in others they show pycnotic nuclei and indistinctly outlined cytoplasm. The latter closely resemble many of the larger colloid masses.

2. Epithelial Portion

a. Pars Anterior

The pars anterior is composed almost entirely of orange-red acidophiles, except for a tongue-like portion adjacent to the nervosa in the median dorsal portion of the gland. The acidophiles are closely packed, showing no definite arrangement into cords or tubules (Pl. II, Fig. 7). They vary in shape from rounded to ovoid to spindle shaped. Their size is also variable. The cytoplasm contains fine orange-red granules. The nuclei vary in size in relation to the size of the cells containing them. Their shapes range from spherical to elongate and their position may be central or apical. Within each nucleus are one or two red-staining spherical nucleoli surrounded by red-staining chromatin granules in a clear hyaloplasm. The tongue-like strip of tissue (Pl. I, Fig. 2) is a sheet several layers thick, roughly "V" shaped, which fits into the uebergangsteil with which it is continuous laterally and ventrally. Its posterior surface is in contact with the pars nervosa from which it is clearly separated by a membrane, and its anterior surface is in contact with the acidophilic part of the pars anterior. Here the delineation is less definite as many of the acidophiles penetrate the tongue-like portion irregularly. Many blood vessels pass through this sheet, enclosed within processes from the nervosa. The cells of this layer bordering the nervosa tend to be columnar while those within the sheet are poorly defined. The cytoplasm has a fleecy appearance and stains a pinkish-gray color. Nuclei are ovoid to spherical, containing a centrally located nucleolus and scattered granular chromatin, both of which stain similarly to the cytoplasm.

b. Pars Intermedia

The pars intermedia is composed entirely of basophiles. Its cytology is variable, the posterior part often showing a large number of basophiles, larger and more clearly defined than the cells of its middle portion, which closely resemble basophiles of the uebergangsteil. The cytoplasm of these larger ovoid to spherical cells ranges from violet to blue and appears homogeneous. Nuclei are roughly spherical, often having indentations which give them a vesicular appearance. They contain granular, faint pink-staining chromatin and a centrally located red-staining nucleolus. The rest of the intermedia cells are smaller and usually indistinctly outlined, their stainability varying from pale gray-blue to a deep blue. Small spherical vacuoles are often present. No special arrangement is apparent in these cells (Pl. I, Fig. 6; Pl. II, Figs. 7, 8). The nuclei are small and ovoid to spherical with well defined membranes in which is a granular chromophobic chromatin surrounding a centrally located red nucleolus. Nervosa processes enclosing blood vessels penetrate the middle region of the pars intermedia after passing through the uebergangsteil, while loose wavy masses of fibers penetrate the dorsal posterior portion directly (Pl. II, Fig. 7).

Besides varying in cell type, the pars intermedia varies in size. In some it is large while in others it is much reduced, forming a thin layer over the uebergangsteil and a small posterior part.

C. Uebergangsteil

The uebergangsteil is the most changeable part of the gland, varying both as to size and as to proportions of its three cell types, basophiles, acidophiles and chromophobes (Pl. I, Fig. 6; Pl. II, Figs. 7, 8). Sometimes single cells or islets of cells lie free among the fibres of the pars nervosa.

The most common type of cell is a large polygonal or round deeply staining carmine red cell with well defined borders. The cytoplasm is filled with coarse carmine-colored granules. The nuclei are spherical to ovoid with a fine granular light red chromatin reticulum surrounding a centrally located brilliant red nucleolus. In some pituitaries these cells were present almost exclusively (Pl. I, Fig. 6). Other glands have an uebergangsteil which is heavily basophilic with a few scattered acidophiles and chromophobes. In such glands the stainable material of the acidophiles is often clumped and peripheral in location. The basophiles closely resemble the acidophiles in size and shape and their nuclei appear identical (Pl. II, Fig. 8). Their cytoplasm is optically homogeneous and varies in staining capacity

from pale gray-blue to deep blue. In some glands basophiles are present only in one or two spherical, centrally located masses which may be exclusively basophilic or may contain some acidophiles also. Chromophobes are smaller, poorly outlined and faintly pink-staining cells, more prevalent, if present at all, in the ventral region of the uebergangsteil. Their nuclei are similar to those of the chromophiles.

II. *Xiphophorus helleri.*

The pituitary of *Xiphophorus* is typically deeper dorso-ventrally than that of *Platypoecilus variatus* and is more concave at its dorsal surface (Pl. I, Fig. 4).

Cell types of the four portions of the gland correspond to those described above with the exception that several small patches of basophiles are usually present in the pars anterior (Pl. I, Fig. 4). These have a small amount of fleecy, indistinctly outlined bluish-gray cytoplasm and nuclei with a coarsely granular, similarly colored chromatin reticulum. They resemble quite closely the cells of the tongue-like portion of the pars anterior.

Another difference from *Platypoecilus* is found in the arrangement of the cells of the uebergangsteil. In *Xiphophorus* cells of this region form a deep mass heavily penetrated by small curving fibrous tracts enclosing blood vessels, which, in median sagittal sections, appear to divide the tissue into rounded islands (Pl. I, Fig. 4). In *Platypoecilus* the uebergangsteil is a thinner, more convoluted layer through which the nervosa processes pass in a more direct manner.

III. *Lebistes reticulatus*

The pituitary of *Lebistes reticulatus* is slightly more elongate and dorso-ventrally flattened than either of the preceding (Pl. I, Fig. 5; Pl. II, Fig. 10). Fewer nervosa processes penetrate the tongue-like portion of the pars anterior than is the case in *Platypoecilus variatus.* The uebergangsteil is more of a convoluted layer than in *Platypoecilus* (Pl. II, Fig. 10), and is heavily penetrated by nervosa processes with their blood vessels. One or two conspicuous masses of basophiles are characteristically present in the central region of the uebergangsteil.

In the pars intermedia of *Lebistes* a broad fibrous tract often reaches from the posterior end of the nervosa, postero-ventrally to the median ventral border of the gland. This is not shown in the figures of *Lebistes* but may be seen in Pl. I, Fig. 3, of *Mollienisia sphenops,* and Pl. II, Fig. 9, *of Limia tricolor.*

Cell types and arrangement are almost identical with those of *Platypoecilus.* However, patches of pale basophiles, similar to those of *Xiphophorus,* are occasionally present in the pars anterior, especially in the Trinidad variety of *Lebistes.*

IV. *Limia tricolor*

The pituitary of *Limia tricolor* is similar in shape to that of *Xiphophorus* (Pl. II, Fig. 9). The pars anterior is heavily penetrated by nervosa processes as in the case of *Platypoecilus.* A broad tract of nervosa fibres cut through the uebergangsteil and pars intermedia to the median ventral surface of the gland as in the case of *Lebistes.*

Cell types correspond to those in *Xiphophorus,* sparse groups of basophiles being characteristically present in the pars anterior.

V. *Mollienisia sphenops*

The pituitary of *Mollienisia sphenops* is different in shape from all the others, being almost perfectly rounded, and flattened dorso-ventrally, (Pl. I, Fig. 3; Pl. II, Fig. 12). Like *Lebistes* and *Limia* it has a broad nervosa tract extending postero-ventrally through the uebergangsteil and pars intermedia to the median ventral surface of the gland. Occasional pale basophiles are found scattered in the pars anterior as in the case of *Xiphophorus,* and are also present along the nervosa processes entering the pars anterior.

The uebergangsteil is more highly convoluted than in *Platypoecilus* and *Lebistes,* and often a linear arrangement of carmine-colored acidophiles forms a border between the nervosa and basophiles and acidophiles of the deeper layer of the uebergangsteil.

VI. *Mollienisia latipinna*

The pituitary of *Mollienisia latipinna* is most similar in shape to that of *Xiphophorus helleri* (Pl. I, Fig. 1). The extension of the infundibular cavity, the recessus hypophyseus, penerates the gland more deeply than in any of the other species. Many nervosa processes with their blood vessels penetrate the tongue-like portion of the pars anterior to be distributed throughout the basophilic part of the pars anterior. Along these processes, and scattered in small groups throughout the mass of acidophiles, are pale basophiles, a condition similar to that found in *Mollienisia sphenops.*

Discussion.

I. Anatomy

Pituitary morphology and histology are fundamentally similar in the six species of poeciliids examined. The glands most closely resemble those of the egg laying poeciliid, *Fundulus,* and the ovo-viviparous poeciliid, *Jenynsia lineata,* as described by Scruggs (1939) and Rojas et al. (1934) respectively.

The infundibular stalk is highly variable among teleosts, being almost non-existant in *Mormyrus* (Stendell, 1914), the pituitary

being held close to the brain, and short in *Fundulus* (Matthews, 1936) and *Carassius auratus* (Bell, 1937, 1938; Levenstein, 1939; Scruggs, 1939). In *Lophius piscatorius* (de Beer, 1926) the stalk reaches its highest development, extending anteriorly for a considerable distance. It may be solid as in *Carassius* or hollow as in *Pungitus* (Scruggs, 1939) and in the poeciliids described here.

No structure comparable to a sella turcica is present in the teleosts. In *Fundulus* (Matthews, 1936) and *Carassius* (Bell, 1938; Levenstein, 1939) the pituitary is protected by the parasphenoid bone. The poeciliid crania of this investigation differ from the above, being of the platybasic type as described by Kingsley (1936). The cranium floor is composed of calcified connective tissue which spreads out laterally over the trabeculae cranii. The pituitary projects ventrally into the hypophyseal fenestra, a depression in the floor of the cranium.

A. Pars Nervosa

The pars nervosa protrudes into the gland at different angles in various teleosts. In *Carassius* (Bell, 1938; Levenstein, 1939) and in *Cyprinus* (Scruggs, 1939) the gland is tilted forward and the nervosa runs anteriorly. In *Ameiurus nebulosus* (Scruggs, 1939) the pituitary is tilted backward and the nervosa runs posteriorly. In the poeciliids investigated here the pituitary is directly beneath the infundibular stalk and the nervosa is directed vertically downward.

A recessus hypophyseus, representing an extension of the infundibular cavity into the nervosa, is present in *Jenynsia* (Rojas et al., 1934), *Fundulus* (Matthews, 1936), and in the poeciliids of this investigation. It is lacking in some teleosts, as *Carassius* (Bell, 1938; Levenstein, 1939) and *Ameiurus* (Scruggs, 1939).

There is also much variation in the number of nervosa processes given off and in their penetration of the various portions of the gland. In *Pseudopleuronectes americanus* and *Cyprinus carpio* (Scruggs, 1939) the nervosa processes are limited exclusively to the pars intermedia; in *Fundulus*, to pars intermedia and uebergangsteil; in *Carassius*, *Jenynsia* and the poeciliids of this report, the nervosa processes go to all parts of the pituitary.

B. Pars Anterior

The proportions, contacts and positions occupied by the various portions of the pituitary in different teleosts are highly variable. In *Carassius* (Bell, 1938; Levenstein, 1939; Scruggs, 1939) and in *Cyprinus carpio* (Stendell, 1914; Scruggs, 1939) the pars anterior is small, dorsal in position and makes contact with the uebergangsteil mostly and the nervosa only in a limited area. In *Notemigonus* (Scruggs, 1939) it is large and anterior in position, making contact with the uebergangsteil and the main portion of the nervosa. In *Ameiurus nebulosus* the pars anterior is small. It is anterior and ventral in position, again making contact with uebergangsteil and nervosa (Scruggs, 1939). In the fishes of the investigation the pars anterior is very large, anterior in position, and touches nervosa, uebergangsteil and pars intermedia. The variations, however, are more apparent than real, being due in large part to a tilting of the pituitary either backward or forward.

A pars anterior has been reported for all teleosts examined except *Esox niger* (Scruggs, 1939) and *Fundulus* (Matthews, 1936). However, in his 1937 paper Matthews decided that the anterior portion of the pituitary, previously called the uebergangsteil, was actually the pars anterior. This was substantiated by Scruggs (1939) on the basis of staining reactions. In the case of *Esox niger* Scruggs found no portion taking a stain like that of the pars anterior of other teleosts; Stendell (1914), however, describes a small pars anterior for *Esox lucius*.

C. Pars Intermedia

The pars intermedia of teleosts has usually been identified by its close relationship with the pars nervosa. Stendell (1914) shows an intimate relationship of the two parts in the primitive *Mormyrus*, while in the higher teleost, *Esox lucius*, the pars intermedia is more posterior in position and retains its association with the nervosa by means of nervosa processes extending out into it. It extends forward ventrally, reaching or nearly reaching the posterior end of the pars anterior, thus surrounding the uebergangsteil ventrally. While Matthews (1937) divides the pituitary of *Fundulus* into only two epithelial portions, a pars anterior and a pars intermedia, Scruggs (1939) shows that the latter portion may be differentiated, by the Dawson & Friedgood (1938) method, into two portions comparable to the pars intermedia and the uebergangsteil of other teleosts. The pituitary of the poeciliids of this investigation compare with that of *Fundulus* as found by Scruggs, having an uebergangsteil adjacent to the pars nervosa and a pars intermedia which surrounds it laterally, ventrally and posteriorly. Connection with the nervosa is direct posteriorly and by means of nervosa processes extending through the uebergangsteil centrally and anteriorly.

D. Uebergangsteil

The uebergangsteil is present in some cyclostomes and all teleosts. It is highly variable in the latter, the simplest condition being found in *Mormyrus* where the

pars anterior, uebergangsteil and pars intermedia are arranged in linear order with no distinct boundaries between them. In *Gasterosteus* (Bock, 1928) connective tissue septa separate the parts. In *Carassius auratus* (Bell, 1938) the uebergangsteil is very large, bordered by connective tissue, and forms most of the anterior part of the gland. In *Esox niger* (Scruggs, 1939) no pars anterior is reported and the uebergangsteil occupies the antero-dorsal region of the gland, being in size, shape and location similar to the pars anterior of *Carassius auratus*. The uebergangsteil of the poeciliids investigated here has no connective tissue septa, is moderately large, and as in most teleosts, lies between the pars anterior and the pars intermedia, a portion of the latter extending over it, however, in the middle region of the gland. It is highly variable in individuals of the same species, being a large deep layer in some, a thinner layer in others. In *Lebistes*, *Platypoecilus* and *Mollienisia sphenops* it tends to be highly convoluted while in the other species examined it is a thicker, less folded layer.

II. Histology

A. Pars Nervosa

Stendell (1914) described the nervosa most completely. It is composed chiefly of neuroglia cells. The infundibular cavity and recessus hypophyseus are lined with primitive ependyma cells having protoplasmic extensions both basally and distally. Bock (1928) confirms these findings in *Gasterosteus*. Through the neuroglia network Stendell describes lymph tracts, blood vessels and connective tissue. Stolon-like nervosa processes extend to all parts of the gland. He interprets the structure of the nervosa as providing the means of absorption of pars intermedia secretions by way of lymph and blood vessels. Colloid masses among nervosa fibres, he believes represent secretion of degenerating pars intermedia cells. Collin (1924), in mammals, Florentin & Weiss (1931), Florentin (1934) and Rojas et al. (1934), in teleosts, hold the same theory of secretion and absorption. The structure of the pars nervosa of the poeciliids investigated here confirms that of the above authors. Nervosa processes ramify throughout all portions of the pituitary, especially the pars intermedia. Colloid masses of varying size and amount are present in the nervosa and pars intermedia. Matthews (1936), Levenstein (1939), Scruggs (1939) and others call attention to masses of colloid in the nervosa. Stendell (1914) also notes many free cells of the pars intermedia lying in the nervosa and believes that they degenerate into colloid. In the present work a number of free cells were seen lying in the nervosa, some appearing degenerate and closely resembling colloid masses.

B. Pars Anterior

The pars anterior of teleosts differs greatly from that of other vertebrates, consisting almost entirely of acidophiles, with sometimes a scattering of basophiles. Entirely basophilic pars anteriors have been reported in the eel, *Cyprinus*, *Esox lucius* and in *Carassius* by Tilney (1911), Stendell (1914) and Bell (1938). However, modern technics have shown these same portions to be predominantly acidophilic (Florentin & Weiss, 1931; Scruggs, 1939; Levenstein, 1939). These discrepancies thus appear to be due to differences in staining technics.

In the present work the pars anterior is almost entirely acidophilic. Occasional single or small groups of pale basophiles may be present, especially in *Xiphophorus*, *Limia* and the *Mollienisias*. Their scarcity precludes any great physiological significance. An additional type of cell, an orange acidophile, is described in some species by Scruggs, using the Dawson & Friedgood technic. These are difficult to interpret since in some species they are the predominant or exclusive type of cell present in the pars anterior while in others a few are present among a background of carmine-staining cells. Also similar orange cells are described in the pars intermedia of some species.

The arrangement of pars anterior cells differs greatly in the various groups of teleosts, varying from a compact condition with no special arrangement as found in the poeciliids in this report to a tubular distribution in which the cells are arranged around lumina, as in the eel (Tilney, 1911) and in the Salmonidae (Scruggs, 1939). An intermediate condition, where the cells are arranged in solid cords, is seen in the cod (Herring, 1908). In those forms in which cord or tubule arrangement is present the cords or tubules are separated from one another by connective tissue septa. Such separation is difficult to discern, or absent in the compact type of gland.

A pale basophilic or chromophobic tongue-like portion of the pars anterior has received little attention; Bock (1928) reports it in *Gasterosteus* and Scruggs refers to it briefly in the Salmonidae, *Fundulus* and *Pungitus*. This portion is present in all the Poeciliidae investigated in the present report, occupying a position in the dorsal half of the gland, between the acidophilic part of the pars anterior and the pars nervosa. It is a layer several cells in thickness, the cells adjacent to the pars nervosa being ependyma-like, oriented with their long axes perpendicular to their contact with the nervosa.

C. Pars Intermedia

Most of the recent work on teleost pituitaries shows the pars intermedia to be composed almost entirely of small pale-staining

basophiles. Scruggs (1939), using the Dawson & Friedgood technic, also found a varying number of orange cells. In *Esox,* Scruggs describes basophiles, orange cells and carmine cells; in *Fundulus,* basophiles, chromophobes and orange cells. Matthews (1936) describes acidophiles of the pars intermedia lining the nervosa processes. These cells, however, seem to belong to the uebergangsteil as described by Scruggs in the same species, rather than to the pars intermedia. The poeciliids investigated in the present report closely resemble *Fundulus* in pituitary structure. In these the pars intermedia, with the Masson stain, is entirely basophilic. The uebergangsteil lies between part of the intermedia and the nervosa, and deep red cells of the uebergangsteil often line the nervosa processes.

The arrangement of cells in the pars intermedia is generally agreed to by all investigators. In *Anguilla* and *Esox* (Stendell, 1914) polygonal cells form a compact stratum traversed by many nervosa processes. Bordering the nervosa processes the cells become cuboidal to columnar, forming an epithelium. Bell (1938) describes the cells of the pars intermedia of *Carassius* as ovoid or polygonal but forming an epithelial layer about the nervosa processes. Scruggs describes the intermedia cells of *Carassius* as indistinctly outlined. In the present work the pars intermedia shows a compact arrangement with polygonal or indistinctly outlined cells. An epithelial arrangement may occasionally be found adjacent to nervosa processes.

The pars intermedia varies both in structure and in the proportion of the gland which it occupies. Rojas et al. describe the disappearance of typical cells in the posterior portion and the appearance of colloid droplets in this location. The same condition is noted in the pars intermedia of the poeciliids investigated here. The dorsal portion of the intermedia is sometimes filled with small colloid droplets and few typical cells are present. Herring (1908) was the first to note such colloid in his work on the cod, and since then Stendell (1914) and many others have called attention to its presence. Scruggs (1939) reports a decrease in the size of the pars intermedia of *Fundulus* in January as compared with June. The pars intermedia of the poeciliids investigated here shows considerable variation in the proportion of the gland which it forms. In some cases a portion of its territory is occupied by large basophiles which appear to belong to the uebergangsteil.

D. Uebergangsteil

The uebergangsteil is composed of acidophiles and basophiles in the eel and in *Mormyrus,* acidophiles predominating in the former and basophiles in the latter (Stendell, 1914). In the stickleback (Bock, 1928) chromophobes and a few basophiles are present. Scruggs (1939) finds no acidophiles in the uebergangsteil of the eel, only basophiles and chromophobes being present. In most of the teleosts examined by him, he reports deep-staining acidophiles and basophiles, and chromophobes. The poeciliids examined here have the three characteristic cell types.

Uebergangsteil cells may form a compact mass with acidophiles and basophiles scattered in groups (Bell, 1937, 1938; Charipper, 1937; Levenstein, 1939; Scruggs, 1939) or a folded epithelial configuration as seen in Scruggs' figures of the Centrarchidae and Poeciliidae. A similar condition is seen in the figures of Matthews (1936), Rojas et al. (1934) and in the poeciliids of this investigation.

Cell type proportions are highly variable in the uebergangsteil of any single species. Bock (1928), Rojas et al. (1934), Matthews (1936) and Scruggs (1939) all call attention to changes in cell types. Matthews finds seasonal changes in the proportionate number of acidophiles and basophiles of *Fundulus.* A particular type of basophile is present in the posterior part of the pars intermedia only at certain times of the year. Scruggs also reports seasonal changes in the *Fundulus* pituitary and describes the migration of large deep-staining basophiles which invade the posterior part of the pars intermedia. In this investigation, large basophiles are present in many specimens in the posterior part of the pars intermedia, similar to those of the uebergangsteil. Also great variability is manifested in percentage of basophiles and acidophiles in different individuals, the uebergangsteil of some being almost entirely basophilic, others almost entirely acidophilic, still others having varying proportions of each. These variations are suggestive of a regulated cyclic variation which may be related to the reproductive cycle. However, cell counts failed to establish any definite relationship between stages of the reproductive and percentage of cell types.

E. Colloid

Herring (1908) first called attention to colloid in the cod pituitary. Stendell (1914) emphasized the functional relationship between nervosa and pars intermedia and postulates two methods of secretion by the intermedia, one by release of minute colloid droplets which are absorbed by the nervosa processes. In the second, intermedia cells wander into the nervosa and disintegrate, forming secretory material which is absorbed by the nervosa. Rojas et al. (1934) and Florentin (1934) likewise describe holocrine secretion in teleost pituitaries. Rojas states that cells of the posterior part of the pars intermedia degenerate and only colloid is left in their place. Matthews (1936) de-

scribes acidophilic colloid in the nervosa which closely resembles the secretory inclusions of the acidophiles of the pars "intermedia" as described by him. In the poeciliids investigated here the posterior portion of the pars intermedia is highly colloidal in many specimens and few cells are present. In others the region is cellular and has little or no colloid. Also single cells or small groups of cells from the uebergangsteil are sometimes present in the nervosa, many of them apparently in the process of disintegration and closely resembling some of the colloid masses. Thus both types of secretion as interpreted by Stendell (1914) are confirmed in this report.

F. Blood Supply

Bock (1928) and Bell (1938) describe poor vascularization in the pituitaries of *Gasterosteus* and *Carassius* respectively. On the other hand, a heavy vascular supply is described in the eel (Tilney, 1911) and *Cyprinus* and *Esox* (Stendell, 1914). The poeciliids investigated here most closely resemble the eel in vascularity. Large vessels in the nervosa radiate outward in all directions within the nervosa processes, and penetrate all parts of the gland.

G. Homologies

Establishment of homologies of the parts of the teleost pituitary with those of mammals is desirable as a prelude to experimental work. Stendell (1914), Charipper (1937), Bell (1938) and Levenstein (1939) homologize the teleost uebergangsteil with the mammalian pars anterior. Levenstein shows two types of chromophobes in the uebergangsteil having Golgi corresponding to those of the acidophiles and basophiles of this portion. This compares with the findings of Addison (1916), Atwell (1929), Severinghaus (1933) and Kirkman (1937) in mammals. The present work shows three types of cells in the teleost uebergangsteil similar to those in the mammalian pars anterior. The pars intermedia of mammals and teleosts occupies a similar position and is almost exclusively basophilic.

The pars anterior of teleosts is interpreted by Bock as a portion unrelated to the pars anterior of higher vertebrates. Charipper (1937) and Bell (1938) compare it with the pars tuberalis of higher forms on the basis of location, cord-like arrangement of cells and basophilic staining reaction. Scruggs (1939), using the Dawson & Friedgood technic, finds the teleost pars anterior to be acidophilic, however, while the pars tuberalis of mammals is chromophobic with the same technic. In the present investigation a tongue-like portion of the pars anterior, occupying a position along the dorso-anterior surface of the nervosa, close to the infundibular stalk and brain, takes a pale basophilic or chromophobic stain. Bock (1928) and Scruggs (1939) describe a similar portion in several teleosts. This portion seems to compare more favorably with the pars tuberalis of higher forms. If this be the case, then the rest of the pars anterior can be considered only as a separate structure characteristic of teleosts and of some cyclostomes.

Summary and Conclusions

1. The pituitaries of the six species of ovoviviparous poeciliids conform, in the presence and general disposition of epithelial and nervous portions, with the structure reported for other teleost fishes. The four portions are the pars anterior, pars intermedia, uebergangsteil and pars nervosa.
2. Boundaries between the parts are established by abrupt changes in cell type rather than by connective tissue septa.
3. The pars nervosa consists of a solid mass of fibrous tissue and neuroglia cells. It occupies the dorsal and central portion of the pituitary gland and sends solid root-like processes to all the epithelial portions. Within the nervosa are varying amounts of colloid, usually violet-colored, large, amorphous masses and some finer acidophilic granules.
4. The pars anterior is composed almost entirely of small orange-red acidophiles which show no special arrangement into cords or nests. A tongue-like strip of faintly basophilic or chromophobic cells separates the acidophilic portion from the pars nervosa throughout the dorsal half of the gland.
5. Only occasional single or small groups of basophiles are present in the pars anterior of *Xiphophorus helleri*, *Limia tricolor*, *Mollienisia latipinna* and *Mollienisia sphenops*, and in a few *Lebistes*.
6. The pars intermedia consists of small faintly-staining basophiles showing no special arrangement. The posterior region is less cellular and more heavily penetrated by fibres from the nervosa and takes a violet color with the Masson stain. The middle portion is heavily cellular and stains light blue. Many small vacuoles but little or no colloid are present in this region.
7. The uebergangsteil is a thick layer of cells surrounding the pars nervosa completely laterally but only on its ventral half anteriorly and posteriorly. It may form a highly convoluted layer typical of *Lebistes* and *Platypoecilus variatus*, or a thicker, less folded layer as in *Xiphophorus helleri*. In all cases many strands of the nervosa penetrate it to reach the middle portion of the pars intermedia. Large granular acidophiles, large and more homogeneous basophiles

and occasional chromophobes constitute the cellular population of this region.

8. There appears to be a regulated variation of the pars intermedia and the uebergangsteil in which the proportions occupied by the two parts varies inversely. Not only do the proportions of the two parts change but also the ratio of basophiles and acidophiles in the uebergangsteil changes. No correlation was established, however, between these changes and stages of the reproductive cycle.
9. The pars anterior, intermedia and nervosa have an abundant blood supply. The uebergangsteil has no direct blood supply, but it is penetrated in many places by strands of the nervosa tissue, each of which ensheathes a blood vessel. The many foldings of the uebergangsteil layer bring most of the epithelial tissue in close contact with the blood vessels. In cases where the uebergangsteil is not convoluted the nervosa processes with their blood vessels show heavy anastomoses with each other, thus dividing the epithelium into many patches or islands bordered by blood vessels.

Bibliography.

Addison, W. H. F.

1916. The Golgi apparatus in the cells of the distal glandular portion of the hypophysis. *Anat. Rec.*, vol. 11, pp. 317-318 (Suppl.).

Atwell, W. J.

1929. Characteristics of the Golgi apparatus in the different types of cells in the anterior lobe of the cat's hypophysis. *Anat. Rec.*, vol. 42, p. 44 (Suppl.).

deBeer, C. R.

1926. The comparative anatomy, histology, and the development of the pituitary body. Oliver and Boyd, London.

Bell, W. R.

1937. Studies of the endocrines of teleosts. I. The morphology of the hypophysis of the goldfish (Carassius auratus L.). *Anat. Rec.*, vol. 70, p. 122 (Suppl.).

1938. Morphology of the hypophysis of the common goldfish (Carassius auratus L.). *Zoologica*, vol. 23, pp. 219-234.

Bock, F.

1928. Die Hypophyse des Stichlings (Gasterosteus aculeatus L.) unter besonderer Beruchsichtigung der Jehrescyklischen Veranderungen. *Ztschr. wiss. Zool.*, Bd. 131, S. 643-710.

Cardoso, D. H.

1934. Relations entre l'hypophyse et les organes sexuels chez les poissons. *C. R. Soc. Biol.*, T. 115, pp. 1347-1349.

Charipper, H. A.

1937. The morphology of the hypophysis in lower vertebrates, particularly fish and amphibia, with some notes on the cytology of the pituitary in Carassius auratus (the goldfish) and Necturus maculosus (the mudpuppy). Cold Spring Harbor Symp. Report, vol. 5, pp. 151-164.

Charipper, H. A. & Haterius, H. O.

1932. The histology of the pituitary of the albino rat in relation to the oestrus cycle. *Anat. Rec.*, vol. 54, pp. 15-27.

Collin, R.

1924. Passage de la colloïde hypophysaire dans la substance cérébrale chez le chien. *C. R. Soc. Biol.*, T. 91, pp. 1334-1335.

Dawson, A. B. & Friedgood, H. B.

1938. The differentiation of two classes of acidophiles in the anterior pituitary of the female rabbit and cat. *Stain Tech.*, vol. 15, pp. 17-81.

Florentin, P.

1934. Histophysiologie comparée de l'hypophyse. L'excrètion de la colloïde hypophysaire chez the Teleostéens. *Ann. de Physiol. et Physico. Biol.*, T. 10, pp. 963-965.

Florentin, P. & Weiss, M.

1931. Etude histologique de l'hypophyse de l'anguille (Anguilla anguilla L.). *C. R. Soc. Biol.*, T. 107, pp. 718-720.

Herring, P. T.

1908. A contribution to the comparative physiology of the pituitary body. *Quart. Journ. Exper. Physiol.*, vol. 1, pp. 261-289.

Houssay, B. A.

1931. Action sexuelle de l'hypophyse sur les poissons et les reptiles. *C. R. Soc., Biol.*, T. 106, pp. 377-378.

Kingsley, J. S.

1926. Outlines of the comparative anatomy of vertebrates. P. Blakiston's Son and Co., Phila.

Kirkman, H.

1937. A cytological study of the anterior hypophysis of the guinea pig and a statistical analysis of its cell types. *Amer. Jour. Anat.*, vol. 61, pp. 233-287.

Levenstein, I.

1939. The cytology of the pituitary gland of 2 varieties of goldfish (Carassius auratus L.), with some reference to variable factors in the gland which may possibly be related to the different morphological types. *Zoologica*, vol. 24, pp. 47-60.

Matthews, S. A.

1936. The pituitary gland of Fundulus. *Anat. Rec.*, vol. 65, pp. 357-369.

1937. The development of the pituitary gland in Fundulus. *Biol. Bull.*, vol. 73, pp. 93-98.

Rasmussen, A. T.

1921. The hypophysis cerebri of the woodchuck (Marmota monax) with special reference to hibernation and inanition. *Endo.*, vol. 5, pp. 33-66.

Rojas, P. L., Castellengo, L. &

Silva Alcorta, M. E.

1934. La hypofisis de Jenynsia lineata (Jen). *Rev. Soc. Argent. de Biol.*, vol. 10, pp. 414-420.

Scruggs, W. M.

1939. The epithelial components of the teleost pituitary gland as identified by a standardized method of selective staining. *J. Morph.*, vol. 65, pp. 187-214.

Severinghaus, A. E.

1933. A cytological study of the anterior pituitary of the rat, with special reference to the Golgi apparatus and to cell relationship. *Anat. Rec.*, vol. 57, pp. 149-176.

Stendell, W.

1914. Die Hypophysis Cerebri. Oppel's Lehrbuch der vergl. Mikr. Anat., Teil 8, S. 1-162.

Tilney, F.

1911. Contribution to the study of the hypophysis cerebri with especial reference to its comparative histology. *Mem. of the Wistar Inst. of Anat. and Biol.*, No. 2, pp. 3-92.

Wolfe, J. H. & Cleveland, R.

1933. Cyclic histological variations in the anterior hypophysis of the albino rat. *Anat. Rec.*, vol. 55, pp. 233-249.

1935. Cyclic histological variations in the anterior hypophysis of the dog. *Z. Zellforsch.*, Bd. 17, pp. 420-452.

EXPLANATION OF THE PLATE.

All material illustrated has been fixed with a modified Helly's fluid, stained with the Masson stain, and cut at 5 micra (except Fig. 3 which was cut at 7 micra).

Plate I.

Fig. 1. Median sagittal section through the infundibular stalk of a female *Mollienisia latipinna*, showing the recessus hypophyseus, nervosa processes entering the pars anterior and other portions of the gland. × 140.

Fig. 2. Median sagittal section through the pituitary of *Platypoecilus variatus* showing pars nervosa and part of pars anterior. Neuroglia cells and fibres are visible in the pars nervosa. The tongue-like portion of the pars anterior is clearly visible. × 610.

Fig. 3. Median sagittal section through the infundibular stalk of *Mollienisia sphenops* showing nervosa tract descending from the posterior region of the pars nervosa to the ventral border of the gland. A patch of basophiles is present in the pars anterior, adjacent to a nervosa process. The convoluted arrangement of the uebergangsteil is apparent. Colloid is abundant both in the pars nervosa and in the posterior region of the pars intermedia. × 250.

Fig. 4. Median sagittal section through the infundibular stalk of *Xiphophorus helleri* showing the four portions of the gland. Small gray patches of basophiles and the gray tongue-like portion can be seen in the pars anterior. Also, small fibrous tracts carrying blood vessels can be seen penetrating the uebergangsteil. × 175.

Fig. 5. Median sagittal section through the infundibular stalk of the pituitary of a female *Lebistes reticulatus* showing the different portions of the gland. The pituitary is more elongate and dorso-ventrally flattened than in the other species. × 300.

Fig. 6. Cross section through the infundibular stalk of *Platypoecilus variatus* showing the sharp demarcation of the uebergangsteil (dark staining cells) from the middle region of the pars intermedia. Colloid bodies and nervosa processes can be seen in the nervosa. × 160.

Plate II.

Fig. 7. Median sagittal section through the infundibular stalk of a male *Platypoecilus variatus*, the four portions of the gland. The pars anterior is composed almost completely of dark-staining acidophiles. In the pars nervosa colloid bodies are seen among the neuroglia cells and fibres. The uebergangsteil is composed of basophiles and acidophiles, some of which invade the pars intermedia ventrally. × 220.

Fig. 8. Median sagittal section through the pituitary of *Platypoecilus variatus*, showing the large acidophiles and basophiles of the uebergangsteil. Below them are the small basophiles of the pars intermedia. × 900.

Fig. 9. Median sagittal section through the pituitary gland of *Limia tricolor*. A tract of pars nervosa fibres can be seen penetrating to the ventral border of the gland. Many colloid masses are present in this tract and in the dorsal posterior region. × 240.

Fig. 10. Cross-section through the infundibular cavity of the pituitary of *Lebistes reticulatus*, showing the highly convoluted uebergangsteil. × 300.

Fig. 11. Cross-section through the mid-region of the pituitary of *Platypoecilus variatus*, showing the pars nervosa surrounded by the uebergangsteil. Colloid bodies are prominent in the pars nervosa. Numerous blood vessels carried in nervosa processes penetrate the uebergangsteil. × 460.

Fig. 12. Cross-section through the infundibular stalk of the pituitary of *Mollienisia sphenops*, showing a part of the pars anterior at the right. Beneath the gland may be seen the hypophyseal fenestra. × 160.